Study of Complexes of C_2- and C_6-dihydroceramides with Transition Metal Ions Using Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS)

Jin-Yi Lim, Avvaru Praveen Kumar, Changdae Kim,† Chuljin Ahn, Young-Jae Yoo, and Yong-Ill Lee*

*Department of Chemistry, Changwon National University, Changwon 641-773, Korea. *E-mail: yilee@changwon.ac.kr*
†Department of Physics, Mokpo National University, Mokpo 534-729, Korea

Received March 21, 2008, Accepted December 26, 2008

The complexes of C_2- and C_6-dihydroceramides with transition metal ions have been investigated by using Electrospray ionization-tandem mass spectrometry (ESI-MS/MS). The formation and fragmentation pathways of several doubly charged cluster ions as well as singly charged cluster ions of C_2- and C_6-dihydroceramides with transition metal ions have studied by ESI-MS/MS in the positive mode. Under ESI conditions, dihydroceramides form singly and doubly charged complexes with transition metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} except Cu^{2+}) with the compositions of $[DHCer+M+2H_2O-H]^+$, $[2DHCer+M+2H_2O-H]^+$, $[3DHCer+M+2H_2O-H]^+$, $[2DHCer+M]^{2+}$, $[3DHCer+M]^{2+}$, $[4DHCer+M]^{2+}$, $[5DHCer+M]^{2+}$, and $[6DHCer+M]^{2+}$ (DHCer = C_2- or C_6-dihydroceramide, M = transition metal ion). The different complexation behavior of copper is responsible for relatively lower affinity of dihydroceramides to copper compared to those of other transition metals. It is also found that in the mass spectrum of the dihydroceramide complexes with copper(II), $[2DHCer+Cu-H]^+$ was observed with considerable intensity as well as $[2DHCer+Cu+2H_2O-H]^+$ due to its different geometry from those of other metals.

Key Words: Transition metal complexes, C_2-dihydroceramide, C_6-dihydroceramide, Electrospray ionization tandem mass spectrometry

Introduction

Ceramides are a family of lipid molecules, found in high concentrations within the cell membrane of cells. Structurally, the ceramides exhibit a long aminoalcoholic chain covalently bound via an amide linkage to a fatty acyl moiety that can vary in length (synthetic short chain ceramides: C_2-, C_4-, C_6-, . . . or natural ceramides: C_{16}-, C_{18}-, C_{20}-, . . .).[1] They are one of the component lipids that make up sphingomyelin, one of the major lipids in the lipid bilayer. Ceramides have recently been identified as key signal molecules which mediate many biological functions such as cell growth, differentiation, senescence, and apoptosis.[2-7] Cell permeable analogs of ceramide, such as C_2-ceramide and C_6-ceramide, have been used to analyze the role of ceramide in intracellular signaling.[8] Apoptotic activities of C_2 ceramide and C_2 dihydroceramide against HL-60 cells are examined because the circumstances around the primary hydroxyl group is important for the apoptotic activity.[9,10] The wide range biological effects of ceramides that depend on cell type, receptors involved, sub-cellular location, and concentration suggest the existence of several downstream targets for distinct intracellular pathways.

Transition metal ions are known to be important in biological, environmental and many other chemical systems. Transition metal complexes are exploited to promote organic synthesis and transition metal cations, especially multiply charged cations, have a pivotal role in enzymatic processes in biological systems.[11] In most papers, the authors describe complexes of monovalent cations with various organic ligands; however, bivalent cations are also a subject of interest. The ESI mass spectrometry is recognized almost immediately after the introduction of this technique and a significant number of papers on this subject have already been published. The first information on the ESI spectra of $[3M+Met]^{2+}$ complex ions is published in 1990 by Katta *et al.*[12] The ESI mass spectrometry has a potentiality for studying weak, non-covalent interactions between biomolecules and metal cations. Structural characterization of ceramides in positive or negative mode has been reported by electrospray ionization tandem mass spectrometry (ESI- MS/MS)[13-15] and also by fast atom bombardment tandem mass spectrometry (FAB-MS/MS).[16,17] Kerwin *et al.*[18] investigated sphingomyelins and fragmentation studies of ceramides in existence of lithium ion have demonstrated by ESI-MS/MS.[19] The analysis of ceramides with high sensitivity and selectivity without prior separation and derivatization has been studied by using ESI-MS.[20,21]

From the literature survey, it can be realize that tandem mass spectrometry is a useful tool for the study of formation and fragmentation pathways of biological metal complexes due to MS^n (multiple-stage tandem mass spectrometry). Fortunately, there are no previous and extensive studies on ceramide complexation with transition metal ions, so we aimed to explore the complexes of ceramides with transition metal ions. In our previous study,[22] we reported the complexes of C_2-ceramide with transition metal cations using ESI-MS/MS, now we wish to report the transition metal complexes of C_2- and C_6-dihydroceramides, similarities and differences in complexation between the C_2- and C_6-dihydroceramides using ESI-MS/MS in the positive mode.

Experimental

Materials and reagents. D-*erythro*-N-Acetyl sphinganine (C_2-dihydroceramide), N-hexanoyldihydro-sphingosine (C_6-dihydroceramide), manganese(II) chloride, ferrous(II) chloride, cobalt(II) chloride, nickel(II) chloride, copper(II) chloride, and zinc(II) chloride were purchased from Sigma Chemical Co. (St.Louis, MO, USA). Methanol of gradient grade (Merck, Darmstadt, Germany) was used for mass spectrometry. Other chemicals and solvents were purchased from Aldrich Chemical Co. (St. Louis, MO, USA) and used without further purification.

The metal complex solutions of C_2- and C_6-dihydroceramides were prepared by mixing the aqueous methanol solutions of transition metal chlorides at concentrations of 1.0×10^{-3} M to 1.0×10^{-5} M and 1.0×10^{-4} M to 10^{-6} M of ceramide solution in methanol with different concentration ratios of transition metal ion and ceramide (1:1 to 1:200) just before the infusion into the mass spectrometer. The samples thus prepared were infused in aqueous methanol (25/75 %) solutions using a syringe pump at a flow rate of 5 μl min^{-1} and the corresponding ions were introduced into the mass spectrometer by electrospray.

Mass spectrometry. All experiments were performed by using an LCQ-Advantage ion trap mass spectrometer (Thermo Finnigan Co., San Jose, CA, USA) equipped with an ESI source. The sample solution was infused into the electrospray interface by a syringe pump at a flow rate of 5 μL min^{-1}. Operation conditions were as follows; spray voltage, 4.5 kV; capillary voltage, 3 V; heated capillary temperature, 200 °C; and sheath gash (N_2), 20 arb. Helium gas admitted directly into the ion trap was used as the buffer gas to improve trapping efficiency and as the collision gas for CID experiments. Tube lens offset voltages were set by using a tune file created by auto tuning of the LCQ on the ion signal of interest if not specified. CID experiments were performed by setting the isolation width between 5 and 10 mass units depending on the species of focus and the activation amplitude at 5 - 25% of 5 V peak-to-peak in resonance excitation RF voltage. All mass spectra recorded were the average of 20 consecutive scans.

Results and Discussion

The structures of C_2- and C_6-dihydroceramides were shown in Fig. 1, (a) and (b), respectively. The complexation of C_2- and C_6-dihydroceramides with transition metal ions was studied by ESI-MS/MS in the positive mode. Fig. 2 is the full-mass spectrum of the complexes of C_2-dihydroceramide with manganese(II) ion. The spectrum includes several complex ions, not only singly charged cluster ions but also doubly charged cluster ions like $[nDHCer(2)+Mn]^{2+}$ (n : ranging from 2 to 6).

Fragmentation of complexes of C_2-dihydroceramide with Mn^{2+}. Prior to MS^n of $[5DHCer(2)+Mn]^{2+}$, we performed MS/MS and MS^n for a variety of ions ranging from $[3DHCer(2)+Mn+2H_2O–H]^+$ to $[6DHCer(2)+Mn]^{2+}$. Among them, MS^n (n = 2~5) spectra for one of the doubly charged ions, $[5DHCer(2)+Mn]^{2+}$ was illustrated in Fig. 3 (Here, 2 in parenthesis indicates the carbon number of fatty acid). The MS/MS of $[5DH\ Cer(2)+Mn]^{2+}$ (m/z 885) gave rise to $[4DHCer(2)+Mn]^{2+}$ ion at m/z 714 by the loss of one C_2-dihydroceramide. The MS^3 of $[4DHCer(2)+Mn]^{2+}$ which was the outstanding ion produced by MS^2 of $[5DHCer(2)+Mn]^{2+}$ yields fragment ion at m/z 543 by the lack of one C_2-dihydroceramide. By MS^4 of precursor ion at m/z 543, there were several product ions such as $[DHCer(2)+H]^+$ at m/z 344, $[2DHCer(2)+Mn]^{2+}$ at m/z 371, $[2DHCer(2)+Mn–H–H_2O]^+$ at m/z 722, and $[2DHCer(2)+Mn–H]^+$ at m/z 740. The important thing to be noticed is that the intensity of $[2DHCer+Mn+2H_2O-H]^+$ was outstandingly fierce by comparison with that of $[4Cer+M]^{2+}$ or $[5Cer+M]^{2+}$ from C_2-ceramide.[22] This difference is made by the fact that a double bond of C_2-ceramide can enhance the possibility of hydrogen bonding and therefore, four or five C_2-ceramides can provide sufficient stability to manganese ion. That was, the difference in sphingoid backbone of ceramide has a substantial influence on the stability of complex ions. The MS^5 spectrum of $[2DHCer(2)+Mn]^{2+}$ (m/z 371) included two singly charged cluster ions $[DHCer(2)+H–H_2O]^+$ at m/z 326 and $[DHCer(2)+Mn–H]^+$ at m/z 397. $[DHCer(2)+H–H_2O]^+$ was generated by protonated C_2-dihydroceramide with lack of one H_2O and reduction of one C_2-dihydroceramide, one manganese, and one H_2O from $[2DHCer(2)+Mn]^{2+}$. While $[DHCer(2)+Mn-H]^+$ was formed by the loss of one C_2-dihydroceramide and hydrogen ion. The different CID energies with 0.81V,

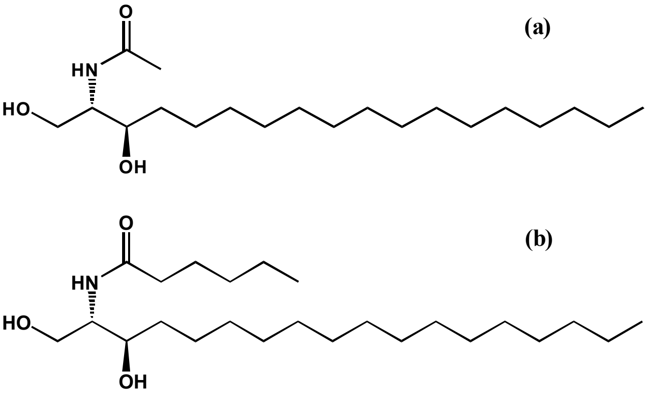


Figure 1. Structures of D-*erythro*-N-Acetylsphinganine (C_2-dihydroceramide) **(a)**; N-Hexanoyl dihydrosphingosine (C_6-dihydroceramide) **(b)**

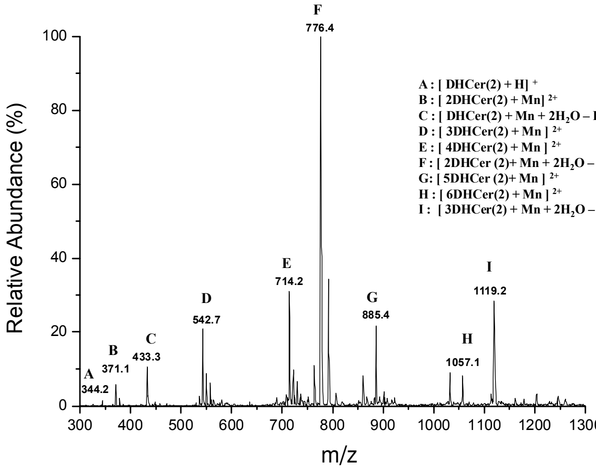
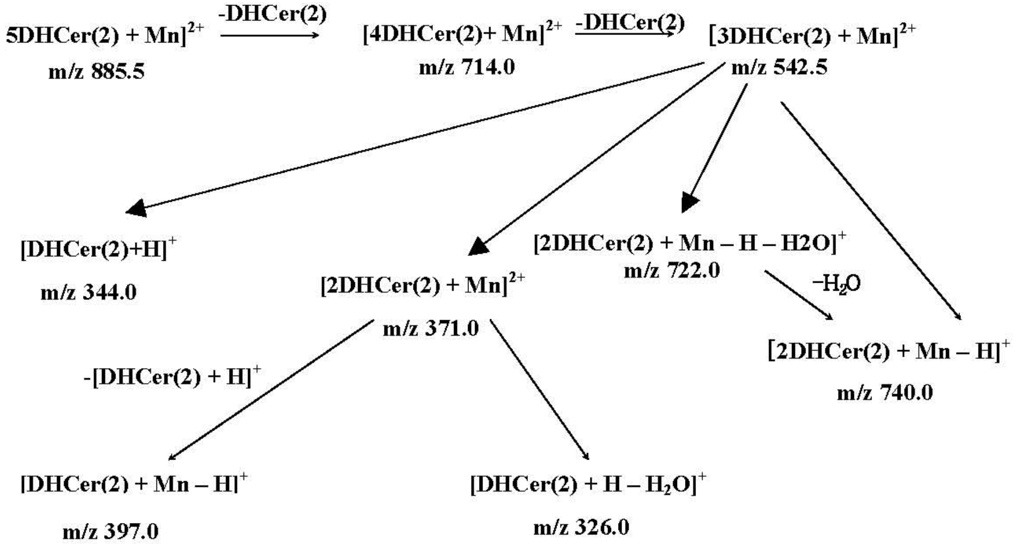
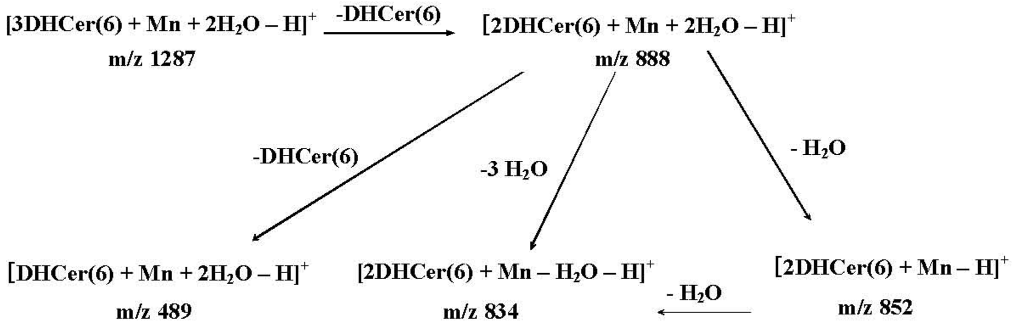


Figure 2. ESI positive ion mass spectrum of aqueous methanol (25/75%) solution containing a mixture of C_2-dihydroceramide ($5.0x10^{-5}$M) dissolved in methanol and manganese(II) chloride ($5.0x10^{-4}$ M) dissolved in aqueous methanol(50/50%) at 20V tube lens offset voltage.



Scheme 1. Proposed CID (MS^n) fragmentation pathway for doubly charged cluster ion, $[5DHCer(2)+Mn]^{2+}$ (m/z 885.4).



Scheme 2. Proposed CID (MS^n) fragmentation pathway for singly charged cluster ion, $[3DHCer(6)+Mn+2H_2O\text{-}H]^+$ (m/z 1287.0).

0.89 V, 0.96 V, and 1.07 V were required to fragment precursor ion, $[nDHCer(2)+Mn]^{2+}$, into several product ions according to the number of C_2-dihydroceramides from five to two, respectively. This fact indicates that $[5DHCer(2)+Mn]^{2+}$ was in the most unstable state than the other doubly charged ions containing two to four C_2-dihydroceramides. The fragmentation pathway of $[5DHCer(2)+ Mn]^{2+}$ was proposed in Scheme 1.

The mass spectrum pattern of C_2-dihydroceramide with manganese(II) ion (Fig. 2) is different from that of C_2-ceramide[22] (Fig. 3 in Ref. 22). The peaks of singly charged cluster ions (Fig. 2) such as $[DHCer(2)+Mn+2H_2O–H]^+$, $[2DHCer(2)+Mn+2H_2O–H]^+$, and $[3DHCer(2)+Mn+2H_2O–H]^+$ as well as doubly charged cluster ions, $[nDHCer(2)+ Mn]^{2+}$ (n = 2~6) were distinguishable from those of C_2- ceramide.[22] Especially, one of the singly charged ions, $[2DHCer(2)+Mn+2H_2O–H]^+$ at m/z 776 was lot more stable than $[4DHCer(2)+Mn]^{2+}$ at m/z 714 or $[5DHCer(2)+Mn]^{2+}$ at m/z 865 whereas in the case of C_2-ceramide,[22] the most eminent ion $[2Cer+Mn+2H_2O–H]^+$ was competitive with $[4Cer+ Mn]^{2+}$ and $[5Cer+Mn]^{2+}$. This is largely because C_2-dihydroceramide does not contain carbon double bond which acts as an acceptor in hydrogen bonds unlike C_2-ceramide. We studied the effect of the structures of C_2-ceramide and C_2-dihydroceramide in the complexation with transition metal ions. The only difference between C_2-dihydroceramide and C_2-ceramide is whether ceramide has double bond connecting C4 to C5 of the sphingoid backbone or not[23]

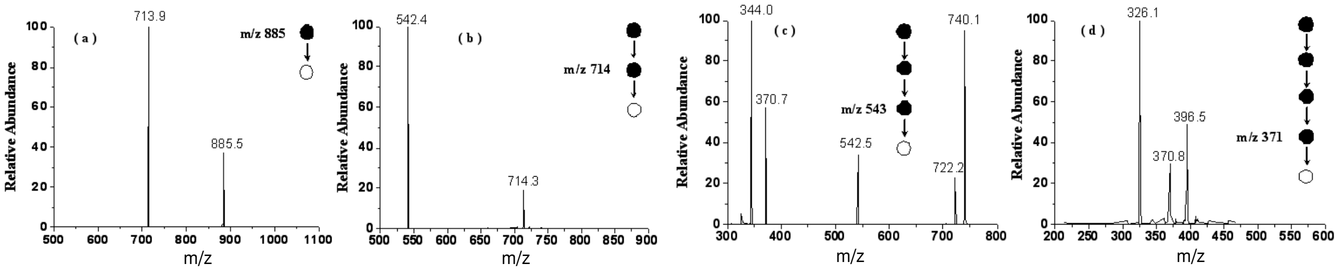


Figure 3. MS^n spectra of doubly charged cluster ion, $[5DHCer (2)+Mn]^{2+}$ (m/z 885) (a) Full-scan MS/MS mass spectrum of $[5DHCer(2)+Mn]^{2+}$ (b) Full-scan MS^3 (885→714→) mass spectrum of $[4DHCer(2)+Mn]^{2+}$ (c) Full-scan MS^4 (885→714→543→) mass spectrum of $[3DHCe r(2)+Mn]^{2+}$ (d) Full-scan MS^5 (885→714→543→371→) mass spectrum of $[2DHCer(2)+Mn]^{2+}$.

and it will be clear that C_2-dihydroceramide does not have carbon double bond connecting through C4 and C5 position which was present at the same position in C_2-ceramide.[22]

Formation and fragmentation of complexes of C_2-dihydroceramide with Cu^{2+}. Fig. 4 indicates the full mass spectrum of C_2-dihydroceramide in the existence of copper ion. As shown in the mass spectrum, the complex ion including C_2-dihydroceramide and copper ion, $[2DHCer(2)+Cu+2H_2O–H]^+$ was dominant. Interestingly, the doubly charged cluster ions corresponding to the formula of $[nDHCer(2)+Cu]^{2+}$ were generated with significantly low abundance. When n is two, the peak of $[2DHCer(2)+Cu]^{2+}$ at m/z 375 was too low to be recognized rather not existed. The fact that the abundance of $[2DHCer(2)+Cu+2H_2O–H]^+$ was remarkable and $[nDHCer(2)+Cu]^{2+}$ has extremely low abundance indicates that two C_2-dihydroceramides allow copper ion to be stable by adding two H_2O molecules and losing a proton. Therefore, it can demonstrate that not $[2DHCer(2)+Cu]^{2+}$ but $[2DHCer(2)+Cu+2H_2O–H]^+$ was observed with notably great abundance. The other feature in the complexation of C_2-dihydroceramide with copper ion is that the peaks of $[DHCer(2)+H]^+$ at m/z 344 and $[2DHCer(2)+H]^+$ at m/z 687 have great intensity. It provides an evidence that enough electron donating groups of C_2-dihydroceramide assist protonated C_2-dihydroceramide itself to be crucially stable. Furthermore, it tells us that the affinity of C_2-dihydroceramide on copper ion is lower than that of manganese ion.

C_2-dihydroceramide did not yield a wide variety of complexes with copper ion comparing with manganese ion. In addition, one of the complexed ions produced, $[2DHCer(2)+Cu+2H_2O–H]^+$ was relatively outstanding ion and having the relative abundance of 100% because copper ion prefers square-planar when complexing with ligands, not to have octahedral geometry. The fact that $[2DHCer(2)+Cu–H]^+$ was also yielded with not low intensity, this might be the other evidence to prefer copper ion for square-planar. The complexation behavior of C_2-dihydroceramide with remaining transition metal ions, Fe^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} is almost similar to that of Mn^{2+}, but there is a difference in intensity for complex ions was observed in all transition metal-C_2-dihydroceramide complexes.

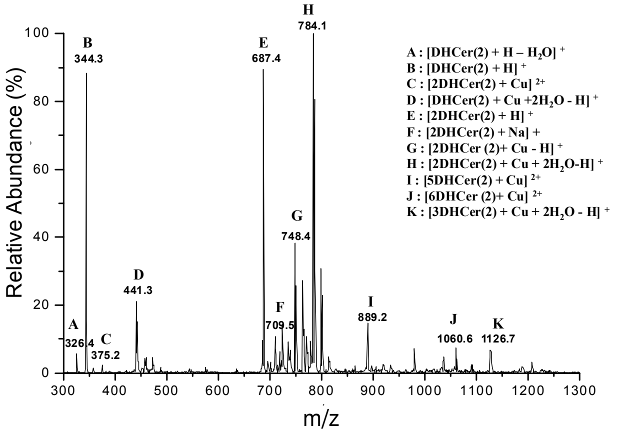


Figure 4. ESI positive ion mass spectrum of aqueous methanol (25/75%) solution containing a mixture of C_2-dihydroceramide ($5.0x10^{-5}$ M) dissolved in methanol and copper(II) chloride ($5.0x10^{-4}$ M) dissolved in aqueous methanol(50/50%) at 20V tube lens offset voltage.

Formation and dissociation of complexes of C_6-dihydroceramide with Mn^{2+}. A series of experiments on C_6-dihydroceramide with transition metal ions were carried out to explore the influence of difference in fatty acid on the complexation with transition metal ions. Overall, the pattern of peaks for eminent ions generated was similar to that of C_2-dihydroceramide. There are various ions of both singly and doubly charged cluster ions; $[DHCer(6)+Mn+2H_2O–H]^+$ at m/z 489, $[2DHCer(6)+Mn+2H_2O–H]^+$ at m/z 888, $[3DHCer(6)+Mn+2H_2O–H]^+$ at m/z 1287, $[DHCer(6)+H-H_2O]^+$ at m/z 382, $[DHCer(6)+H]^+$ at m/z 400, $[2DHCer(6)+Na]^+$ at m/z 821, $[2DHCer(6)+Mn]^{2+}$ at m/z 427, $[3DHCer(6)+Mn]^{2+}$ at m/z 626.5, $[4DHCer(6)+Mn]^{2+}$ at m/z 826, $[5DHCer(6)+ Mn]^{2+}$ at m/z 1025.5, and $[6DHCer(6)+Mn]^{2+}$ at m/z 1225 (Here, 6 in parenthesis indicates the carbon number of fatty acid). The most abundant peak of the spectrum was $[2DH Cer(6)+Mn+2H_2O–H]^+$ at m/z 887, the intensities of singly and doubly charged species of C_6-dihydroceramide were higher than those of C_2-dihydroceramide, and when compared to C_2-ceramide[22] the peak pattern and the intensities of singly and doubly charged species are different. The ratio of the peak intensity for $[2DHCer(6)+Mn+2H_2O–H]^+$ to that of $[nDHCer(6)+Mn]^{2+}$ (n = 4 or 5) was reduced as compared with that of C_2-dihydroceramide, this is due to the influence of steric effect to the electronic effect of two C_6-dihydroceramides was relatively severe over C_2-dihydroceamide. In other words, the electronic effect of $[nDHCer(6)+Mn]^{2+}$ (n = 4 or 5) neutralizes the steric effect of those ions. As the number of C_6-dihydroceramides complexed with manganese ion was above three, we estimate the surplus C_6-dihydroceramides interact with manganese ion, but not directly rather secondarily through the other C_6-dihydroceamides which have already combined. However, when the number of C_6-dihydroceramide is seven, $[7DHCer(6)+Mn]^{2+}$, the peak at m/z 1424 was not shown because the electronic effect of the seventh C_6-dihydroceramide was no longer able to the influence on manganese ion to be stabilized.

Fragmentation of complexes of C_6-dihydroceramide with Mn^{2+}. In order to confirm the composition of complex ions of C_6-dihydroceramide in presence of manganese ion, and to monitor the fragmentation pathways of these species, MS/MS and MS^n of those ions have performed. One of the singly charged species, $[3DHCer(6)+Mn+2H_2O–H]^+$ at m/z 1287, was fragmented into $[2DHCer(6)+Mn+2H_2O–H]^+$ at m/z 888 by the loss of one C_6-dihydroceramide. MS^3 spectrum included $[2DHCer(6)+Mn–H]^+$ at m/z 852, $[2DHCer(6) +Mn–H_2O–H]^+$ at m/z 834, and $[DHCer(6)+Mn+2H_2O–H]^+$ at m/z 489. Although the MS^4 and MS^5 were also examined, the spectra does not illustrate because those are too simple with the lack of one H_2O. The fragmentation pathway of $[3DHCer(6) +Mn+2H_2O –H]^+$ at m/z 1287 was proposed in Scheme 2. MS/MS of $[6DHCer(6)+Mn]^{2+}$ at m/z 1225 yields a predominant fragment ion at m/z 1025.5 corresponding to the formula of $[5DHCer(6)+Mn]^{2+}$, by the loss of one C_6-dihydroceramide. $[4DHCer(6)+Mn]^{2+}$ at m/z 826 was found in MS^3 spectrum of $[5DHCer(6)+Mn]^{2+}$, of which the precursor ion was $[6DHCer(6)+Mn]^{2+}$. MS^4 of $[4DHCer(6)+Mn]^{2+}$ produces the doubly charged ion,

$[3DHCer(6)+Mn]^{2+}$ at m/z 626.5 by the removal of one C_6-dihydroceramide. The MS^5 spectrum of the precursor ion, $[3DHCer(6)+Mn]^{2+}$ was fragmented from $[4DHCer(6)+Mn]^{2+}$, consists of singly charged ions such as $[DHCer(6)–H_2O+H]^+$ at m/z 382, $[DHCer(6)+H]^+$ at m/z 400, $[2DHCer(6)+Mn–H_2O+H]^+$ at m/z 834, and $[2DHCer(6)+Mn+H]^+$ at m/z 852 together with $[2DHCer(6)+Mn]^{2+}$ at m/z 427.

Formation and fragmentation of complexes of C_6-dihydroceramide with Cu^{2+}. The full mass spectrum of C_6-dihydroceramide (5×10^{-5} M) in the existence of copper ion (5×10^{-4} M) was not so different with C_2-dihydroceramide. Surprisingly, even though the intensity of $[DHCer(6)+H]^+$ was also considerably high, the dominant peak in the spectrum was protonated C_6-dihydroceramide, $[DHCer(6)+H]^+$, not one of the complexed ions. The rationale behind this is that the synergy between steric effect and electronic effect of C_6-dihydroceramide was produced to make $[DHCer(6)+H]^+$ to be the most stable form. C_6-Dihydroceramide forms a wide variety of complex ions such as $[DHCer(6)+H–H_2O]^+$ at m/z 382, $[DHCer(6)+H]^+$ at m/z 400, $[2DHCer(6)+Cu]^{2+}$ at m/z 431, $[DHCer(6)+Cu+2H_2O-H]^+$ at m/z 497, $[2DHCer (6)+H]^+$ at m/z 799, $[2DHCer (6)+Na]^+$ at m/z 821, $[2DHCer (6)+Cu-H]^+$ at m/z 860, $[2DHCer(6)+Cu+2H_2O-H]^+$ at m/z 896, $[5DHCer(6)+Cu]^{2+}$ at m/z 1029, $[6DHCer(6)+Cu]^{2+}$ at m/z 1229, and $[3DHCer(6)+Cu+2H_2O-H]^+$ at m/z 1296. Through the fact that $[2DHCer(6)+Cu-H]^+$ was found with remarkable abundance as well as $[2DHCer(6)+Cu+2H_2O-H]^+$, the preference of copper ion for square-planar is confirmed. Moreover, the ratio of metal complex ions to C_6-dihydroceramide adduct not including copper ion is lower than C_2-dihydroceramide, due to the steric effect of fatty acid from C_6-dihydroceramide. Since the affinity of C_6-dihydroceramide for copper ion is not stronger than manganese ion, some peaks relating copper ion were seen with rather trivial intensity like $[2DHCer(6)+Cu]^{2+}$ (When the tube lens offset voltage of 10 V was applied and the abundance of this ion was nearly below 5 %). The complexation of C_6-dihydroceramide with other metal ions like Fe^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} is not mentioned since the complexation with those ions was not much different from that of Mn^{2+}.

Conclusions

In the present work, the complexes and their fragmentation pathways of C_2- and C_6-dihydroceramides with transition metal cations (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) were explored by ESI-MS/MS. All of the above mentioned metal ions complex with C_2- and C_6-dihydroceramides to form doubly charged cluster ions as well as singly charged cluster ions containing metal ion and C_2- or C_6-dihydroceramides. Although extreme distinction in the full mass spectrum between C_2-dihydroceramide and C_6-dihydroceramide in existence of transition metal is not seen, the most high abundant peaks of C_6-dihydroceramide, $[DHCer(6)+H]^+$ and $[2DHCer (6)+H]^+$, which are not complexed to copper, were observed. While the most dominant peak of C_2-dihydroceramide, $[2DHCer(2)+Cu+2H_2O–H]^+$, was the complexed ion including copper. The reason for the difference in complexation with copper between C_2-dihydroceramide and C_6-dihydroceramide is that the steric effect due to the difference in carbon number of fatty acid in dihydroceramides. That is, the steric effect of C_6-dihydroceramide outweighs the electronic effect of C_6-dihydroceramide. For copper(II), the most common coordination numbers are 4, 5 and 6, but tetragonal distorted octahedral geometries is not by far different from square-planar while the other metal ions prefer the octahedral geometry.[24] In sum, the unique mass spectrum of copper(II) in the complexation with ceramide results from low ceramide's affinity on Cu(II) and distinctive coordination geometry. Through the experiments performed in this paper, the fact that the tandem mass spectrometry is a rapid, sensitive, and suitable method to investigate the complexation of several ceramides with transition metal ions is proved.

Acknowledgments. The authors gratefully acknowledge the support by the Korea Research Foundation (Grant No. KRF-2007-J00903) and Changwon National University (2007).

References

1. Kolesnick, R. N.; Goni, F. M.; Alonso, A. J. *Cell Physiol.* **2000**, *184*, 285.
2. Jayadev, S.; Liu, B.; Bielawska, A. E.; Lee, J. Y.; Nazaire, F.; Pushkareva, M. Y.; Obeid, L. M.; Hannun, Y. A. *J. Biol. Chem.* **1995**, *270*, 2047.
3. Hannun, Y. A.; Luberto, C. *Trends Cell Biol.* **2000**, *10*, 73.
4. Kolesnick, R. N.; Krönke, M. *Ann. Rev. Physiol.* **1998**, *60*, 643.
5. Riboni, L.; Prinetti, A.; Bassi, R.; Caminiti, A.; Tettamanti, G. *J. Biol. Chem.* **1995**, *270*, 26868.
6. Venable, M. E.; Lee, J. Y.; Smyth, M. J.; Bielawska, A.; Obeid, L. M. *J. Biol. Chem.* **1995**, *270*, 30701.
7. Pena, L. A.; Fuks, Z.; Koksnick, R. *Biochem. Pharmacol.* **1997**, *53*, 615.
8. Okazaki, T.; Bielawska, A.; Bell, R. M.; Hannun, Y. A. *J. Biol. Chem.* **1990**, *265*, 15823.
9. Fillet, M.; Bentires-Alj, M.; Deregowski, V.; Greimers, R. *et al. Biochem. Pharm.* **2003**, *65*, 1633.
10. Shikata, K.; Niiro, H.; Azuma, H.; Ogino, K.; Tachibana, T. *Bioorg. Med. Chem.* **2003**, *11*, 2723.
11. Silverman, R. B. *The Organic Chemistry of Enzyme-Catalyzed Reactions*; Academic Press: New York, 2000.
12. Katta, V.; Chowdhury, S. K.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 5348.
13. Hsu, F. F.; Turk, J. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 558.
14. Raith, K.; Neubert, R. H. H. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 935.
15. Han, X. *Anal. Biochem.* **2002**, *302*, 199.
16. Ann, Q.; Adams, J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 260.
17. Ann, Q.; Adams, J. *Anal. Chem.* **1993**, *65*, 7.
18. Kerwin, J. L.; Tuininga, A. R.; Ericsson, L. H. *J. Lipid Res.* **1994**, *35*, 1102.
19. Hsu, F. F.; Turk, J.; Stewart, M. E.; Downing, D. T. *J. Am. Soc. Mass. Spectrom.* **2002**, *13*, 680.
20. Cremesti, A. E.; Fischl, A. S. *Lipids* **2000**, *35*, 937.
21. Gu, M.; Kerwin, J. L.; Watts, J. D.; Aebersold, R. *Anal. Biochem.* **1997**, *244*, 347.
22. Lim, J. Y.; Kumar, A. P.; Lee, Y. I. *Eur. J. Mass Spectrum.* **2008**, *14*, 87.
23. Kok, J. W.; Karakashian, N. M.; Klappe, K.; Alexander, C.; Merrill, A. H. Jr. *J. Biol. Chem.* **1997**, *272*, 21128.
24. Shen, J.; Brodbelt, J. *J. Mass Spectrom.* **1999**, *34*, 137.